Epidermis: An Attractive Target Tissue for Gene Therapy

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Important advances have been made within the past several years in understanding diseases at the molecular and cellular levels, which may enable the application of somatic gene therapy to a wide variety of genetic and acquired diseases. The initial clinical trials involving somatic gene therapy have demonstrated that gene transfer into human subjects can be performed safely and with public acceptance. This review focuses on use of the epidermis as a target tissue for gene therapy and assesses various delivery systems for both *ex vivo* and *in vivo* approaches. In addition, we discuss candidate diseases that may be amenable to epidermal gene therapy and the advantages of employing transgenic mouse model systems to test the efficacy of a given gene therapy prior to clinical trials. ***Key words: keratinocyte/delivery/vectors/bioreactor. J Invest Dermatol 103:63S–69S, 1994***

Of all the recent advances in novel pharmacologic or surgical therapeutics, it is the potential for gene therapy that has captured the interest and imagination of both the scientific and public communities. Gene therapy is defined simply as the introduction of exogenous gene into a host cell (transduction) to achieve a therapeutic benefit [1]. Although often thought applicable to the treatment of inherited diseases, this approach is also being applied to acquired diseases, ranging from cardiovascular disorders to cancer [1,2]. The ability to attempt this form of therapy derives from the progress made over the past decade in recombinant DNA technology and cell biology. By employing these techniques, important advances have been made in understanding diseases at the molecular and cellular levels, in identifying factors that regulate gene expression, and in methods for gene transfer. Yet whereas there remain significant problems facing practical gene therapy (reviewed in [2]), numerous protocols have been proposed and approved for clinical trials with surprising rapidity. Most notably, the treatment of malignant melanoma by infusion of tumor infiltrating lymphocytes engineered to excrete large amounts of tumor necrosis factor [3]; and treatment of adenosine deaminase (ADA) deficiency, a rare genetic disease where patients lack the ADA gene, by reintroducing cultured T lymphocytes that have been transduced with a normal copy of the ADA gene [4]. Both these protocols require an *ex vivo* approach, ie somatic cells are cultured *in vitro*, the gene introduced, and then modified cells returned to the body. Therefore, it would be most advantageous to develop *in vivo* gene therapy and numerous experimental techniques are currently under intense investigation in many laboratories.

In this chapter, we will discuss the use of the epidermis as a target tissue for gene therapy and assess the various delivery systems available for both *ex vivo* and *in vivo* approaches. We will also highlight the use of endogenous promoters that may result in differentiation specific or inducible expression, and evaluate some of the diseases that may be treated by an epidermal approach. Finally, we will consider the safety aspects and the advantages of employing transgenic mouse model systems to test the efficacy of a given gene therapy prior to clinical trials.

ADVANTAGES OF EMPLOYING THE EPIDERMIS FOR GENE THERAPY

There are several attractive reasons for considering the epidermis as a target tissue for gene therapy. First and foremost is the accessibility of this tissue. Aside from the ease of administering a potential *in vivo* therapeutic approach, the ability to monitor the treatment site and, if necessary, easily remove the genetically modified area, is an advantage for safety considerations not readily available for other tissues. The epidermis, being a stratified epithelium, is continuously renewed throughout the adult life span through the proliferative stem cells. Therefore, the potential to transduce the epidermal stem cell creates the very attractive possibility of achieving long-term treatment. A stratified epithelium also allows the possibility of targeting gene expression to either differentiated or proliferative cells, depending upon disease requirements. Another distinct advantage is that the biology of the epidermis is relatively well characterized, at both the cellular and molecular levels. The conditions for culture of primary human keratinocytes have been defined ([5], below), and many of the proliferative and differentiation specific genes have been characterized [6]. The regulatory sequences of some of these genes, e.g., the keratins, have already been employed to express a variety of exogenous genes in the epidermis of transgenic mice [7–9] and are therefore readily adaptable for gene therapy applications. The use of specific epidermal promoters as vectors to express exogenous genes, results in both high levels of expression and keratinocyte specificity.

Epidermal gene therapy is envisaged to be generally applicable to the treatment of a wide variety of diseases. Obviously, it will be employed to treat acquired or genetic diseases of the epidermis, especially where expression of a single gene product could alleviate the symptoms. Thus, a hyperproliferative skin disease, such as psoriasis, could be treated with an inhibitor of keratinocyte growth, e.g., transforming growth factor β (TGFβ) expressed from a keratin-based targeting vector [10]. Alternatively, a genetic skin disease, such as epidermolytic hyperkeratosis, that is caused by a point mutation in either kerain K1 or K10 (reviewed in [11]), could be treated by vectors designed to inhibit expression of the mutant allele, or

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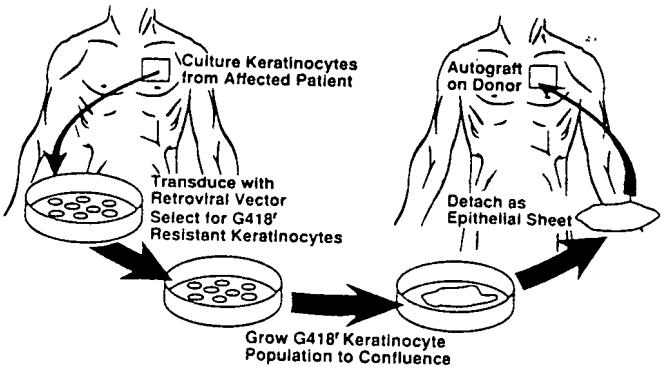

Figure 1. A schematic of an *ex vivo* approach to epidermal gene therapy. Keratinocytes are cultured *in vitro* from a small skin biopsy. Once sufficient numbers are obtained, the keratinocytes are reseeded at a density that ensures high numbers of proliferating cells for transduction by a retroviral vector that contains the therapeutic gene of interest as well as the neomycin resistance gene. A brief selection in the neomycin analog G418 ensures the survival of transduced cells only, which are then grown to confluence. Treatment of cultures with dispase releases the keratinocytes as an epithelial sheet that is then returned to the patient as an autograft.

potentially, by over-expression of the normal keratin gene to ameliorate the condition (below). As the epidermis is known to secrete a variety of cytokines [12] and growth factors [13], it may become a useful bioreactor, geared to the secretion of gene products that have a local or systemic effect. This has an obvious potential for the treatment of diseases such as hemophilia (below). Conversely, the epidermis could be engineered to act as a metabolic waste disposal unit for circulating toxins. Indeed, introduction of the ADA gene [4] into keratinocytes, which could then metabolize circulating deoxyadenosine, has been proposed for the treatment of ADA [14]. Finally, there is an emerging epidemic of melanoma and squamous cell carcinoma [15]; thus development of gene therapy protocols for skin cancer is an important goal.

EX VIVO AND *IN VIVO* GENE DELIVERY SYSTEMS

The method of gene transfer is currently the biggest hurdle-limiting application of this technology. Gene delivery must be highly efficient, otherwise the non-transduced cells limit the effectiveness of the genetically altered cells. Also, a high efficiency is required to achieve transduction of the keratinocyte stem cells that are present in low numbers. It is desirable to transduce sufficient numbers of stem cells to minimize repeated treatments and thereby increase cost effectiveness. Once into a cell, the exogenous gene must integrate into the host genome, or alternatively exist as a stable, replicating episome similar to papilloma viruses [16]. If this is not achieved, then the introduced gene would be eventually lost due to either degradation or deletion following repeated mitoses. Stem cell transduction and gene integration are geared to achieve long term persistence of the therapeutic gene and, to date, although some results are encouraging [14], this has yet to be fully achieved. There are basically two avenues of gene delivery: an *ex vivo* approach, which to date has been the most intensively studied and is likely to be applicable in the near future, and development of an *in vivo* approach that is the more desirable, but will require greater study. Each technique has advantages and disadvantages and specific requirements for optimal gene delivery. As detailed below, a major effort is underway to develop and refine these techniques.

***Ex vivo* Gene Therapy: Transduction of Cultured Keratinocytes by Retrovirus Gene Delivery** The *ex vivo* approach requires culture of the patient's cells that are transduced *in vitro*, and then the genetically altered cells are returned to the patient **(Fig 1)**. Often, the requirement for primary cell culture is considered one of the disadvantages of this approach. However, the epidermis is ideally suited to an *ex vivo* approach, as the culture conditions have been clearly defined [5]. Keratinocytes are usually co-cultured using irradiated fibroblasts as a feeder layer [5]. Using this system, large numbers of keratinocytes can be generated from a small skin biopsy ([5] **Fig 1**). When approaching confluence, the cultured keratinocytes can be detached as an epithelial sheet and returned to the donor as an autologous skin graft **(Fig 1)**. This technique has been widely employed in the treatment of severe burns [17]. The fact that such grafts can persist for several years [18], suggests that a significant number of stem cells are present in these cultures. *In vitro* studies confirm this idea, and have identified populations of keratinocytes with varying growth potential [19]. These have been termed paraclones [19], which have the greatest proliferative potential, and holoclones, or transit amplifying cells [20], which have only limited proliferation capacity. Moreover, recent work on these proliferative populations suggests that the stem cell residing in the paraclone populations [19] may express characteristic surface markers such as specific integrins [21]. Thus, it may be possible to potentially separate stem cells (eg by fluorescence-activated cell sorting [FACS]) *in vitro*, prior to introduction of the gene [21]. This would be a significant advantage over an *in vivo* approach, as greater numbers of transduced stem cells would increase the chances of long term therapeutic effects.

Another advantage of the *ex vivo* approach over the *in vivo*, is the potential to apply selection techniques for transfected cells to ensure integration **(Fig 1)**. However, the usual methods of *in vitro* gene transfer (electrophoresis, polybrene, lipofection, calcium phosphate) followed by selection, are ineffective due to low transfection frequencies. Therefore, the logical alternative is retroviral mediated gene transfer and, to date, most clinical trials are based on the use of replication defective murine retroviruses [1]. What makes them so advantageous is that not only do nearly 100% of replicating cells become infected [22], but also the DNA becomes stably integrated into the target genome, insuring its presence in the descendants of each infected cell.

Retroviruses are RNA viruses that reverse transcribe their genome into DNA that then integrates into the host chromosomes [23]. Thus transcription of viral genes, RNA processing, and encapsidation are regulated by both viral genes and host cell factors [23]. Retroviral mediated gene transfer requires two components. The first is the packaging cell line [24], which contains a retroviral genome that produces the viral proteins for virion formation, but contains a defective signal for encapsidation and therefore cannot produce infectious virus particles. A retroviral vector is then transfected into this packaging cell line. The gene of interest replaces the viral protein coding genes, but the vector retains functional encapsidation and replication signals. Any combination of promoter and gene can be inserted into the retroviral vector, which also usually contains a selectable marker, such as the neomycin resistance (neo) gene that confers resistance to the neomycin analog G418. The packaging cell line then assembles this vector into an infectious viral particle. Thus, it is the nature of the packaging cell line that determines the infectivity range, with ecotrophic virus being centered on one species only, whereas the more useful amphotrophic varieties can infect most mammalian species, which allows animal testing prior to use in humans. Usually, the size of the gene cloned into these vectors is limited to 7 kb or less, necessitating the use of cDNAs, otherwise viral titers become reduced [25].

Using this *ex vivo* approach, several laboratories have pioneered the use of retroviral mediated gene transfer into proliferating keratinocytes towards a strategy of using the epidermis as a bioreactor ([26,27], and below). Another advantage for using the epidermis is that the packaging fibroblast cell line can also be employed as the feeder layer for the cultured keratinocytes, thus increasing transduction frequencies. Recent results are encouraging, and suggest that epidermal stem cells are not only readily transduced, but that these genetically altered cells are long lived. In one study, single clones of transduced cells were expanded to 10^9 cells prior to senescence [22] and, in another, canine keratinocytes, transduced with the neo gene, were shown to persist as a graft for up to 120 d [14]. Furthermore, canine keratinocytes cultured from these grafts re-

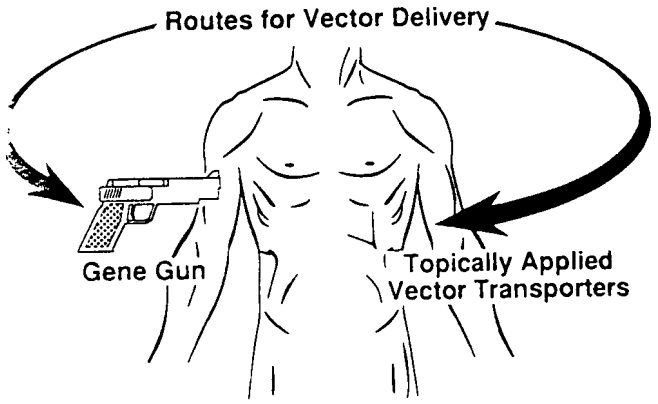

Figure 2. **A schematic of an *in vivo* approach employing the epidermis.** Several simple *in vivo* routes exist for most tissues, e.g., systemic injection or injection directly into the tissue. The epidermis however easily lends itself to very novel approaches. The first method utilizes particle bombardment with the gene gun. This method involves coating DNA to gold particles approximately 1–3 microns in diameter, and projecting these particles directly into the skin at high velocity [45]. The second approach utilizes topically applied vector transporters, i.e., gene cream, which, depending on its make-up, may or may not require the exposure of poliferative basal cells by tape stripping or penetration enhancers.

tained expression of the neo gene, suggesting that a gene regulated by viral promoters could remain active for long periods in keratinocytes ([14] see below).

Although retroviral vectors are to date the mainstay of cell transduction, several features may limit their usefulness. The most significant is the potential for generating replication-competent virus, although this problem has been reduced with the advent of improved vectors and packaging cell lines [24]. Another safety consideration is the potential for insertional mutations, due to the random integration of the viral construct into important loci producing unfavorable consequences. Moreover, it is difficult to predict the influence of surrounding sequences at the integration site on the expression characteristics of the therapeutic gene. Retroviral virion particles are also relatively unstable in comparison to other viruses and purification often leads to loss of infectivity [2]. In addition, amphotrophic retroviruses are readily inactivated *in vivo* in primates [28], which has significant implications for *in vivo* approaches (below) using retroviral mediated transfer. Furthermore, retroviruses cannot be made synthetically, they must be produced by living cells, which would be costly on a large scale. Some of these problems can be overcome by using alternative viral vectors (reviewed in [2]), although these are less well developed. For instance, the use of adeno-associated virus (AAV), which is known to specifically integrate into a region of human chromosome 19, would minimize the deleterious effects of insertional mutations [1,2]. Human papilloma viruses (HPV) are also potential candidates for gene therapy vectors, as they specifically infect stratifying epithelia and have the advantage of replicating as an episome [16]. Indeed, a vector based on the related bovine papilloma virus has been successfully tested in murine cells [29]. With the advent of successful HPV replication in cultured human keratinocytes [30], it is anticipated that HPV derived vectors will be employed for *ex vivo* gene therapy.

In Vivo Gene Therapy: Viral and Non-Viral Delivery Systems

The accessibility of the epidermis makes it an attractive target for development of *in vivo* gene therapies **(Fig 2)**. An *in vivo* approach has significant advantages over the *ex vivo* systems not only from a commercial view point, with elimination of costly culture systems, but from a clinical point of view also, where the culture requirements would limit patient treatment to advanced medical centers. The goal then is to achieve the highly desirable genes in a bottle for affordable therapy in basic clinical settings. Currently, the major hurdle to *in vivo* gene therapy is the problem of getting the DNA into the vicinity of the target cell prior to transduction. Various methods have been proposed, ranging from ballistic projectiles, the so called gene gun **(Fig 2)**, to simple injection, either systemically or directly into a specific tissue. For the epidermis, topical application of a vector transport system (i.e., a gene cream) is also an exciting but speculative alternative.

The majority of *in vivo* studies to date center on the use of retroviral vectors for gene transfer. Retroviral vectors have been injected directly into either diseased tissue, e.g., to treat malignant tumors [31], or into the systemic circulation, with a view to express genes in the liver [32]. For an *in vivo* epidermal approach, to maximize the infectivity, it may be necessary to expose the basal cells, e.g., via tape stripping of the superficial layers or scarring and simply apply a retroviral vector topically **(Fig 2)**. To date, we have had reasonable success using this technique on mouse skin to introduce the β-galactosidase (βgal) reporter gene (Greenhalgh *et al*, manuscript in preparation). There are three significant problems. The first is that viral titers higher than 10^7 cfu cannot be achieved, thereby limiting infection efficiency. Secondly, cells must be dividing for infection [33]. This is a serious limitation for an *in vivo* retroviral mediated approach because it also requires that the stem cells be undergoing mitosis [33]. On occasion, however, this facet may be advantageous, especially in the treatment of cancer where a suicide gene is introduced that kills the rapidly dividing cancer cells, but only a minimum of normal cells ([31], see below). Thirdly, several studies have shown the gradual inactivation of expression from viral promoters [4,34], even though the vector DNA remains present [35]. Thus again, even if stem cells can be transduced with retroviral vectors at reasonable frequencies, maintenance of long term effects may require multiple treatments.

A second viral delivery system that has attracted a great deal of recent interest is based on adenoviruses [36]. These vectors have the advantage in that they do not require the target cell to be mitotically active for infection and, moreover, extremely high titers can be generated (10^{11} cfu). Also unlike retroviruses, adenoviruses are relatively stable and can thus be concentrated without significant loss of infectivity [2]. Adenoviruses are DNA viruses with a tropism for respiratory and oral keratinocytes. Their 35 kb DNA genome encodes early and late genes, and the elimination of E1 region containing the EIA and EIB genes, renders the adenovirus replication defective [36]. Thus, the E1 region of an adenovirus vector becomes an obvious site for the insertion of an exogenous promoter/gene cassette. To propagate the adenovirus vector it is first transfected into a human 293 kidney cell line, which expresses both the EIA and EIB proteins, and these act in trans to package the adenovirus vector DNA producing the infectious viral particles. Cellular infection by adenovirus is achieved by a receptor mediated endocytosis, followed by disruption of the endosome and nuclear localization of the adenovirus genome, where it remains extrachromosomal [1,2]. To date, an adenovirus vector has been employed to transfer the cystic fibrosis conductance regulator protein to the respiratory epithelium of cotton rats with impressive results [37], and this has rapidly led to clinical trials with the hope of alleviating the pulmonary manifestations of cystic fibrosis. In preliminary experiments, we found that topical infection of scarred, murine epidermis with an adenovirus vector carrying a βgal reporter produced high numbers of infected cells, and even direct injection of this adenovirus vector into tumor tissue resulted in an impressive number of cells expressing βgal (Greenhalgh *et al.*, manuscript in preparation). The major problem with adenovirus based vectors is the lack of viral integration [2]. Thus, any potential therapeutic benefit of expression is limited to the differentiation transit time of the infected keratinocytes. For long term gene therapy, this failure to integrate is a significant drawback, but the adenovirus hexon proteins remain useful for receptor mediated delivery (below).

Although the main emphasis in gene delivery centers on viral transfer, a great deal of interest is being generated in the development of non-viral systems. Such methods are attractive because they do not impart any viral sequences into the cell and lend themselves to large scale manufacturing. There are several approaches available

including, receptor-mediated transfer, liposomal transfer, ballistic transfer and injection of DNA/calcium phosphate coprecipitates [1,2,38]. Receptor mediated transfer is achieved by linking the DNA to a protein ligand via polylysine. These complexes can then be injected into the blood stream and will target cells that express the protein ligand receptor, allowing the complex to gain entry into the cell by endocytosis. To date, the liver system has been the most characterized model, being targeted via genes conjugated to transferrin [39]. The major advantage of this process is that it results in a very efficient method of gene transfer necessary for systemic delivery. Unfortunately, a significant drawback is that once endocytosis has occurred, the endosomes are transported to lysosomes where the DNA is subjected to degradation reducing transduction frequencies. To overcome this problem several groups have cleverly utilized the observation that adenoviruses disrupt endosome function [40,41], and have combined this adenovirus function with the receptor mediated transfer technique. With this approach plasmid DNA (of any size) is linked to an appropriate ligand via polylysine and this complex is then conjugated to the adenovirus by an antibody specific to the adenovirus hexon protein [40,41]. Thus, any cell expressing a desired receptor, can be transduced with high frequency and, following endocytosis, the adenovirus protein disrupts the endosome before any DNA damage occurs. As only adenovirus proteins are required, no viral genes are transmitted. This technique has increased transfection efficiency over 1000 times. Indeed, in a recent study by Christiano and co-workers [41], 100% of primary hepatocytes were transduced *in vitro*. *In vivo* experiments are eagerly anticipated to test long term persistence and expression of introduced genes. For the epidermis one can envisage the use of specific ligands that target only keratinocytes, e.g., via keratinocyte growth factor [42]. In the future it may be possible to use this technique with ligands that are specific for keratinocyte stem cell receptors.

The accessibility of the epidermis is also ideal for the application of liposomal and ballistic gene transfer techniques **(Fig 2)**. Liposomal/DNA complexes have been widely used *in vitro* and are capable of direct gene transfer *in vivo* [43]. Currently, a clinical trial that involves liposome mediated transfer of a major histocompatibility gene directly into tumors to enhance immunologic tumor regression is in progress [44]. Liposomes are associated with a minimal toxicity [43] and function by fusing to the cell membrane and releasing DNA into the cytoplasm. Here there is no specificity to the type of cell transduced, therefore specific expression must rely on the promoter employed (see below). By choosing the appropriate lipid for the liposome DNA complex and a second for penetration of the hydrophobic cutaneous layer, one may generate an epidermal gene cream for topical vector delivery **(Fig 2)**. Such delivery systems are still in their infancy, however, the ballistic approach has already been employed to achieve expression in skin [45]. In this regime, DNA coated microscopic gold particles are accelerated by an electric discharge into living cells. An advantage here is that this gene gun approach requires minimal manipulation of the patient or target site. Because this is a physical procedure, it is also indifferent to the target cell type or its properties.

EXPRESSION OF EXOGENOUS GENES: VIRAL AND ENDOGENOUS PROMOTERS

An important consideration for gene therapy is the promoter/enhancer cassette chosen to regulate the expression of the therapeutic gene. Usually, a high level of expression is required and, for safety considerations, it is advantageous to restrict expression to the epidermal keratinocytes. In addition, inducible genetic elements could be introduced into a composite cassette to enable regulation of dosage. High levels of transcription have been achieved through the use of viral promoters such as those of retroviruses or the cytomegalovirus (CMV) [25]. These promoters are excellent for transient expression at high levels and have been chosen for the treatment of neoplasms [31]. One problem with viral promoters is their down regulation in mammalian cells [34,35], and, therefore, even in systems where persistence of the exogenous gene was observed [14,35] expression levels were reduced [14] and eventually disappeared [35]. However, this was not the case for gene bombardment into rat skin, where dermal cells had extended levels of expression from the CMV promoter [45]. This suggests the possibility that viral promoter inactivation may be dependent upon cell type, means of delivery and the nature of the viral promoter itself. It has also been shown that non-viral promoters can be silenced when situated downstream from a viral promoter [34].

These problems can potentially be overcome by using mammalian promoters. Because several genes have been characterized with epidermal-specific expression, a variety of promoters are available for targeting exogenous gene expression to the integument. Thus, not only can promiscuous expression be prevented in a non-target tissue, but expression can be directed to specific epidermal strata and appendages. In particular, use of specific keratin gene control elements gives a significant targeting flexibility [6]. Depending upon therapeutic requirements, it may be desirable to target basal cells, thus a K14-based vector would be useful [8]. Alternatively, it may be necessary to restrict expression to suprabasal cells, e.g., by employing a K10-based promoter [9]. Where high levels of expression are required (i.e., where the epidermis is employed as a bioreactor), the truncated, HK1-based vector developed in our laboratory may be useful since it has expression in both basal and differentiated cells [7]. Moreover, this is epidermal specific [7], whereas other keratins are expressed at other sites [6,8,9], which may not be desirable. For inducible promoters/vectors, the K6 keratin is an attractive candidate, as its expression is induced by a variety of stimuli including wounding, tape stripping, and retinoid application [6]. Of course any one of the keratin based vectors could become steroid or retinoid sensitive by including the appropriate genetic elements [46].

DISEASES AMENABLE TO EPIDERMAL GENE THERAPY

Correction of Inherited Skin Disorders Of the genetic diseases known in man, about 5% have been associated with a skin phenotype [47], but only in a few of these diseases have the molecular defects been defined. One reason for the limited progress in this area is that many skin disorders display similar clinical symptoms yet are genetically very heterogeneous. Recently, several groups have identified the genetic defect underlying epidermolytic hyperkeratosis (EHK) and epidermolysis bullosa simplex (EBS) (reviewed in [11]). EBS and EHK have been common features, including intra-epidermal blistering due to cytolysis of keratinocytes. Ultrastructurally, these diseases are characterized by a collapse of the keratin filament network, which aggregates around the nucleus as a perinuclear shell or as cytoplasmic clumps. In EBS, the collapse of the filament network is limited to the cells of the basal layer whereas the suprabasal cells are uninvolved. Given the observation that truncated keratins expressed in cultured cells resulted in a perturbed intermediate filament network [48], researchers focused attention on the keratins of EBS and EHK patients. For EBS, point mutations were found in the coding regions of the genes for K5 and K14, and for EHK, we and others have identified mutations in the highly conserved regions of both K1 and K10 (reviewed in [11]).

What then are the options for a gene therapy approach? Obviously, the ideal therapy would result in repair of the mutated gene by homologous recombination. Unfortunately, this is not likely to be practical in the near future. One approach, therefore, would be to inhibit expression of the mutant allele. This may be achieved using antisense RNA or ribozymes expressed from K5/K14-based vectors for EBS or K1/K10-based vectors for EHK. Alternatively, overexpression of the normal protein may be efficacious. A major limitation of these approaches is the requirement for transducing high numbers of cells, including an adequate proportion of stem cells. However, given the high transduction efficiency of receptor mediated/adenovirus protocols (above), one can certainly expect a significant therapeutic, if temporary, benefit from transducing 50% of

the cells in a given lesion. As inherited skin diseases become more fully understood, the development of animal models that mimic the disease will be vital to help address the efficacy of putative treatments [49].

Treatment of Skin Cancer The incidence of skin cancer approaches that of all other malignancies combined [15]. Although there are viable surgical procedures that readily eliminate skin neoplasms, gene therapy would be a useful alternative, especially for tumors that occur at difficult to treat locations, those that are unusually large, and those that are metastatic. There are several ways to employ gene therapy for neoplastic disease, including melanoma. They fall into two basic categories: 1) a generic therapy, where an exogenous gene enhances immunologic surveillance or induces tumor cell death; and 2) specific therapies, where the gene(s) responsible for the neoplasm are targeted for modulation. Of the methods to attack the tumor per se, an *ex vivo* approach to produce a tumor vaccine can be taken. Here tumor cells can be transduced with, eg vectors expressing tumor necrosis factor [3] or cytokines (e.g., interleukin 2 (IL-2), interleukin 4 (IL-4) or granulocyte/macrophage–colony-stimulating factor (GM-CSF)) to elicit a more vigorous immune response upon introduction back to the patient. Direct *in vivo* transduction however is probably more useful, and in this case a retrovirus-mediated transduction may be the ideal means to achieve the introduction of anti-tumor genes. This takes advantage of the fact that retroviruses only infect dividing cells. Thus, using this approach, normal quiescent cells would not be affected. Also, by using a keratinocyte or melanocyte specific promoter, the toxic effect of a suicide gene would also be more restricted to tumor cells. The problem is that simple injection of virus may be insufficient to transduce all tumor cells. This can be alleviated somewhat by injecting the viral producer cells themselves to improve transduction efficiency [31]. In initial *in vivo* testing in animals, scientists have utilized the herpes simplex virus thymidine kinase (HSV-TK) gene as a suicide gene. The HSV-TK protein converts the nucleotide analogue, gancyclovir, into a toxic molecule and as a tumor cell dies it releases this toxin to other non-transduced cells, ie the bystander effect. Thus, brain tumors in rats were shown to regress completely when HSV-TK packaging cells were injected into the tumor followed by systemic injection of gancyclovir for 5 d [31]. This approach, using a melanocyte specific tyrosinase promoter coupled to HSV-TK, also successfully inhibited experimental murine melanomas [38].

The more specific approach is dependent on identifying the crucial genes involved. Carcinogenesis is a multistage process involving cooperation between oncogenes (normal genes becoming activated), and tumor suppressor genes (normal genes becoming inactivated or altered in function). In skin, especially on sun exposed areas, two genes would seem to be prime candidates for intervention. UV-induced point mutations have been detected in the ras^{Ha} oncogene [50] and the p53 tumor suppressor gene [51]. These can be early events present in benign lesions, thus presenting an opportunity for therapeutic intervention prior to overt malignancy. Overexpression of the normal p53 gene may be efficacious, possibly inducing apoptosis [52]. For ras^{Ha}, dominant negative mutants that interact only with the activated oncogenic ras^{Ha} p21 protein in membrane signaling have been described [53]. Thus, over-expression of these mutants would allow therapy to be restricted to tumor cells expressing an activated ras^{Ha} oncogene. As transgenic model systems for carcinogenesis are developed that increase our understanding of carcinogenesis *in vivo* [7–9,54–57], new avenues for gene therapy intervention will undoubtedly arise.

Employing the Epidermis as a Bioreactor for Disease Treatment In addition to its function as an external barrier, the epidermis also secretes a variety of factors, eg vitamin D_3, growth factors and cytokines, that act at both local and distant sites (see [58] for a recent review). This raises the possibility that the epidermis could be used as a bioreactor to produce proteins for systemic release to treat disease. At this time it is not clear whether all proteins secreted by the epidermis can achieve systemic distribution. This critical feature may depend upon the protein size, type (hydrophobic/hydrophilic), and the requirement for peptide signals to cross cell or tissue barriers. Human keratinocytes have already been shown to secrete biologically active proteins following retroviral transduction and grafting onto nude mice. One of the first studies introduced human growth hormone (hGH [26]), unfortunately, whereas hGH protein was detected at the graft site, no circulating protein was detected. This may have been a consequence of the insensitivity of the assay [26], as another study demonstrated that large molecules such as apolipoprotein E (apo E), could be secreted into the bloodstream by grafted human keratinocytes [59]. Indeed, in a recent study testing the possibility of using the epidermis as a bioreactor for the treatment of hemophilia B, factor IX was secreted into the bloodstream following transplantation of transduced human keratinocytes onto nude mice. However, long term expression was not sustained in the circulation. Thus, functional use of the capacity of the epidermis to act as a bioreactor awaits significant progress in developing/maintaining high levels of secretion from keratinocytes *in vivo* and understanding the kinetics of dermal transport.

CONCLUSIONS: SAFETY AND FUTURE PROSPECTS

The safety considerations for gene therapy must be compared to any potential benefit. For retroviral transduction in particular (reviewed in [60]), the possibility of generation of replication competent virus is a serious threat, one that has already resulted in development of T-cell lymphomas in Rhesus monkeys [61]. For this reason, all retroviral protocols should include assays to assess for helper virus, and new packaging lines that are designed to minimize this leakage [24]. Avoiding this method of transduction eliminates the problem, and furthermore, use of DNA receptor-mediated transduction completely eliminates the need for viral genes. However, for nearly all transduction techniques, insertional mutagenesis occurs, with the potential for deregulation of endogenous genes, e.g., oncogenes or tumor suppressors. Only by maintaining the transduced DNA as an episome can this be avoided. Given these potential problems, it is immediately obvious that the epidermis has an easily applied, inherent safety technique, i.e., removal of the treatment area/graft site. In addition, relevant model systems must be developed to directly test the delivery and efficacy of a particular gene therapy *in vivo* prior to clinical trials in humans. Currently testing is limited to *in vitro* assays employing primary human keratinocytes, and these studies have reported that there are no obvious deleterious effects (immortalization, transformation) of introducing a retroviral vector [62]. Although this is encouraging, an *in vitro* assay, even including a nude mouse graft analysis, may not fully predict the *in vivo* result. For instance, retroviral TGFα introduction *ex vivo* into primary keratinocytes gave no obvious effect in nude mouse grafts [63], but in transgenic mice, epidermal over-expression of TGFα resulted in hyperplasia and papillomas [8,56]. Thus, the ability to target gene expression to the epidermis of transgenic mice provides a powerful tool to assess the safety considerations of a particular gene therapy. Also, as outlined above for the epidermis, several transgenic mouse models of diseases already exist [7–10,49,54–57] that represent ideal opportunities to test both delivery and efficacy of a novel gene therapy.

The great potential for gene therapy to treat genetic and acquired diseases is revealed in the speed by which the field, and indeed clinical trials, have evolved. It can only be a matter of time before gene therapy is widely applied. To achieve viable epidermal gene therapy, advances in *in vivo* transduction are required to maintain stable, long term expression. The transduced gene needs to be able to integrate into a safe non-critical site within the genome or be maintained as a replicating episome, and its expression needs to be responsive, when necessary, to extracellular (topical or systemic)

signals. Thus, whereas in this decade gene therapy is in its infancy, in the next century a whole new meaning could be attached to the term designer genes.

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